

Effect of Probiotics on the Metabolic Pathways of Warburg Effect in Cancer Model

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Abstract: Cancer disease is one of the major health problem in most of the countries with the increased incidence of mortality. New mechanism has existed over the cancer development through glycolytic pathway with several genotypic and phenotypic metabolic alterations and biochemical abnormalities is called Warburg effect. Expression of Warburg effect occurred by transcription factors such as HIF-1, STAT3, FOXM1, KLF4, GATA, HSF1 and c-Myc to stimulate the gene activation for transcription and translation. Activation of this factors regulate the signaling pathways like PI3K/Akt/mTOR, Wnt signaling, EGFR signaling etc and glycolytic enzymes like HK, GLUT, LDH and PDK. As the result the mitochondrial function was decreased by inhibiting the pyruvate entering in to TCA cycle and no TCA cycle available to enter into mitochondrial function. Probiotic bacteria exerts a beneficial effects towards tumor suppression, down regulation of glycolytic enzymes and factors implicated in the development of cancer and up regulation of the immune system.

Keywords: Warburg effect, Cancer, Probiotics.

1. Introduction

Cancer ranks the second most common cause of death among worldwide. Numerous type of cancer has been affecting the whole organs of human body. Metabolic abnormalities is one of the root cause of tumor formation and growth. Metabolic alteration in glycolytic pathway was happened through converting end product pyruvate to lactate in presence of oxygen. This process termed as Warburg effect [1], [2]. The abnormal expression of glycolytic pathway leads to affect the cellular process which promote abnormal cell proliferation, and tumor growth. Warburg effect was regulated by several transcriptional factors, glycolytic enzymes, signaling molecules and oncogenes [3]. Understanding the metabolic reaction and pathway of Warburg effect was useful in future for targeting cancer treatment.

2. Transcriptional Regulation of Warburg effect

Transcriptional factors consist of wide number of protein involved in gene expression control to regulate cellular processes. The action of factors is to silence or enhance the specific genes for DNA binding. Most cancerous cell have increased the activity of transcription factor which promote the Warburg effect easily through arresting and altering enzymes,

genes, signaling molecules etc in metabolic pathway. Various transcription factors like HIF-1, STAT3, FOXM1, KLF4, GATA, HSF1, c-Myc were assimilate to cause tumor. Hypoxia-inducible factor-1 (HIF-1) has two different subunit (HIF-1 α , HIF-1 β) regulate oxygen level concentration in cellular process through binding with VHL gene [4]. Loss or mutation of VHL gene involved in oxygen sensing mechanism and increase the expression of HIF-1 [5]. The up regulation of HIF-1 alters the glycolytic enzyme through inducing pyruvate dehydrogenase kinase (PDK) which phosphorylates and inactivates PDH that resulting in increased lactate production through Lactate dehydrogenase (LDH) enzyme secretion. The effect largely affect the mitochondrial respiration by arresting pyruvate entering in to TCA cycle [6]. Root cause of generating Reactive oxygen species is by up regulation of HIF-1 and VHL gene mutation. HIF-1 associated with activating c-Myc oncogenic transcription factor can directly regulate over expression of almost all the genes involved in glycolysis, including GLUT1, glucose phosphate isomerase (GPI), PFK1, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), triosephosphate isomerase (TPI), phosphoglycerate kinase 1 (PGK1), and enolase (ENO1) which shifts glycolysis toward lactate production as opposed to mitochondrial metabolism [7]. STAT3 (Signal transducer and activator of transcription-3) factors over expressed specifically in many solid tumors which significantly alter the cell cycle components with enhancing the cyclic-dependent kinases (D, E and A) [8], [9]. The activate STAT3 encoded with cytokines and growth factor thereby stable feed forward loop develop between normal cells and tumor cells, hence inflammatory response promoted to support tumor growth [10]. FOXM1 act as a major impact on promoting Warburg enzymes through reprogramming the glucose metabolism by binding directly to LDHA, glucose transporter 1 (GLUT1) and hexokinase 2 (HK2). Down regulation of FOXM1 reverse the expression of LDHA, HK2, GLUT1 and prevents the tumor formation [11]. Expression of zinc finger transcription factor KLF4 (Kruppel-like factor 4) promotes mitochondrial metabolic fusion and dramatically change physiological function and cause somatic cell reprogramming [12]. The role of GATA transcription factors is lineage determination during cell development and function. Loss of this GATA transcription factors correlates with morphological

transformation [13]. Heat shock transcription factor 1 (HSF1) are well known in inducing the generous amount of stress causing genes to stimulate the reactive oxygen species. The up regulation of stress related genes leads to reprogramming of energy metabolism which promotes Warburg effect [14], [15].

3. Glycolytic enzymes and the Warburg effect

Glycolytic enzymes regulate the major function of glycolysis. Cancer cell drive the glycolytic pathway from converting pyruvate to lactate through lactate dehydrogenase enzymes. Transcriptions factors inhibit the Pyruvate dehydrogenase activity through pyruvate dehydrogenase kinase (PDK), which has been activated. So, PDK stimulate the Lactate dehydrogenase (LDH) to convert Lactate instead of pyruvate. LDH arrest the pyruvate metabolism entering in to TCA cycle, mitochondrial respiration and nicotinamide adenine dinucleotide (NADH) to (NAD⁺) [16], [17]. Studies proved that knockdown of LDH reverse glycolytic metabolism in preventing cancer development. LDH and PDK act as a major key regulator in promoting Warburg effect. The rate-limiting step for glucose metabolism is the glucose uptake which is facilitated by glucose transporters enzymes at the cell membrane such as GLUT1-4 [18]. Glucose transporters enzymes (GLUTs) mediate the transport of glucose in the intracellular and extracellular level of cell. In cancerous condition GLUT1 and GLUT2 activation were up regulated through the HIF-1 and VHL gene mutation. GLUT3 expression are related to mutation or dysregulation of tumor suppressor gene p53. The condition leads to accumulation of reactive oxygen species to inhibit the glycolytic pathway and result in cell death of immune cells [19], [20].

4. Metabolic Pathway of Warburg effect

Signaling pathway molecules act as a communication process to coordinate various cellular action and function. Cell receive signal and information from the signaling molecules to activate and regulate cellular metabolism. High rate of glucose and lactate production directly alters and dysfunction the signaling molecules to induce Warburg effect. Various signaling pathway has a crucial role in developing cancer. WNT/beta-catenin pathway stimulates PI3K/Akt/mTOR pathway which induce Warburg effect through activation of HIF-1 α . Partially WNT/beta-catenin suppresses pyruvate oxidation in the mitochondrial metabolism. β -catenin acts as regulator of glucose and glutamine metabolism. Expression of WNT/beta-catenin pathway degrade the β -catenin and react through LRP5 and RAC1 to activate mTORC2 and AKT pathway resulting in up regulation of key glycolytic enzymes [21], [22], [23], [24]. The specific mechanism of PI3K-Akt-mTOR pathway regulates the metabolism of cellular building blocks and control the metabolic homeostasis via regulation of the expression or translocation of metabolic genes associated with expression of LDHA, HK-II and GLUTs. The over expression of PI3K-Akt-mTOR pathway stimulate glucose

consumption and metabolism by regulating pyruvate to lactate which increased cell proliferation and apoptosis of normal cells [25], [26], [27]. Fructose 1, 6 bisphosphate directly enhance the EGFR signaling pathway expression. The effect increases the lactate excretion and cancer cell immune escape through inhibition of cytotoxic T-cell activity [28], [29]. These findings implied that cancer signaling pathway regulates not only intracellular metabolic pathway but also extracellular immune escape function. The over expression of erbB-2 proto oncogene stimulates the activation of Ras Signaling pathway and coupled to the epidermal growth factor receptor in breast cancer cell lines [30].

5. Role of Probiotics on Warburg Effect

Probiotics are the beneficial microorganism which modify the intestinal micro flora. It has positive effects on health by acting defense wall of host immune function and inhibition of tumor cells when used as dietary supplement [31]. Most of the Probiotic are genera of *Lactobacillus* and *Bifidobacterium* bacteria. Many studies in *Lactobacillus* and *Bifidobacterium spp* has efficiently proved in preventing proliferation and promote apoptosis in various cancer cell lines. Probiotic has been gaining much attention towards anti-Warburg due to ability of binding carcinogens, modulate the Warburg inducing substances and molecules. *Lactobacillus gasseri* and *Lactobacillus jensenii* bacteria has the inhibit effect on the viability of cervical cancer cells through regulation of cell cycle related genes and up regulate the E-cadherin for inhibiting cancer migration [32]. Anti-Warburg effect through *lactobacilli* against the HIF-1 resulted in anti-proliferative effect accompanied by down regulation of autophagy genes (ATG14 and BECN1) and oncogenes (HPV E6) [33]. The expression of HIF-1 α , HIF-1 β and downstream of genes were strongly suppressed by triterpenoidcorosolic acid (CA) isolated from *saliva syriaca* has cytotoxicity against the cancerous cell in hypoxia condition. CA has a remarkable anti-cancer activity in hypoxia through targeting HIF-1 [34]. Probiotic *Lactobacillus plantarum* and bioactive compounds extracted from *Aesculus hippocastanum* increases TNF- α level and exert a preventive effect on colon carcinogenesis [35]. Biologically active substance isolated from mushroom plant (*Leucopaxillus giganteus*) has also possess several bioactivities including anti-tumor effect in cervical cancer. Clitocine from *Leucopaxillus giganteus* induce apoptosis in HeLA cell lines by activation of caspase-3, -8, and -9, up-regulation of Bax. Polysaccharides isolated from *Lactobacillus acidophilus* has regulate the expression of interacting protein of Bcl-2 and cycle protein of cell division [36]. *Lactobacillus casei* extract has reported significantly reduced the activation of mTOR and NF- κ B signaling pathway to induce apoptosis through caspase-3 expression in gastric cancer cell lines [37]. *Lactobacillus delbrueckii* also proved to be efficient in inhibit of cancer cell proliferation and induce cancer cell death through the caspase 3-dependent pathway in colon cancer cells [38].

Lactobacillus pentosus and *Lactobacillus plantarum* induce the cell arrest of G1 phase through down regulation of mRNA level of cyclin genes such as A, B1, B2 and E [39]. *Bifidobacterium adolescentis* has ability to stimulate the production of nitric oxide (NO), hydrogen peroxide, and cytokines, such as interleukin (IL)-6 and TNF- α in macrophage cell lines to regulate immune modulation and cytotoxic to various cancer cells [40]. Combination therapy of most representative probiotic *Lactobacillus plantarum* and chemotherapy drug of 5-fluorouracil (5-FU) induced anti-cancer mechanism in colorectal cancer by inactivating the wnt/ β -catenin signaling pathway and activate of caspase-3 for cell death [41]. Dietary intake combined with *Lactobacillus acidophilus* and *Bifidobacterium animalis subsp. Lactis* inhibit colorectal carcinogenesis by increasing anti-oxidative capacity and enhance the colonic expression of p53 and Bax to induce apoptosis [42].

6. Conclusion

Over all it shown that, Warburg effect plays a major role in causing cancer disease. Targeting specific pathway proteins of Warburg effect will give promising result to cancer treatment. Understanding the regulation of glycolytic pathway in cancer brings new idea in cancer therapy. Many studies proved that probiotics has the capability of arresting the major expressions of Warburg molecules like HIF-1, caspases and signaling pathways. Molecular events and mechanistic studies also indicated that probiotics have the capacity in inhibiting the expression of Warburg effect and act as an efficient anticancer agent.

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